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- (74) Agents: **VAN MALDEREN, Eric et al.**; Office Van Malderen, Place Reine Fabiola, 6/1, B-1083 Brussels (BE).
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- (71) Applicant (for all designated States except US): **BELOVO** [BE/BE]; Egg Science and Technology, Industrial Area, 1, B-6600 Bastogne (BE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **REMACLE, Claude** [BE/BE]; Rue de Limoy, 69, B-5101 Loyers (BE). **LIGNIAN, Jacques** [BE/BE]; Benonchamps, 100, B-6600 Bastogne (BE). **ERPICUM, Thomas** [BE/BE]; Rue de la Jeunesse, 73, B-4100 Seraing (BE). **DE MEESTER, Fabien** [BE/BE]; Rue du Luxembourg, 46, B-6900 Marche (BE). **COUCKE, Luc** [BE/BE]; Vijfse weg, 158, B-8790 Waregem (BE). **SIM, Jeong** [CA/CA]; 6508-127 Street, Edmonton, Alberta T6H 3X1W (CA). **SCHMIDT, Christian** [BE/BE]; La Comté, 5, B-6690 Vielsam (BE).
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(54) Title: **EGGS WITH BALANCED LIPID COMPOSITION**

(57) Abstract: The present invention is related to an egg obtained from a domesticated bird, in particular a layer, having a lipid fraction balanced in seed and green plant-type  $\omega 6$  and  $\omega 3$  fatty acids according to the ratio of seeds plant-type  $\omega 6$  fatty acids / green plant-type  $\omega 3$  fatty acids =  $1:1 \pm 10\%$  and having a lipid fraction is balanced between polyunsaturated and saturated fatty acids according to the ratio of polyunsaturated / saturated fatty acids =  $1:1 \pm 10\%$ . The present invention is also related to a feed composition of exclusive vegetarian origin and suitable for poultry and a method for obtaining such egg from said poultry animals.

5

## EGGS WITH BALANCED LIPID COMPOSITION

Field of the invention

10 [0001] The present invention relates to wild- or  
game-type eggs having an improved balanced lipid  
composition and which are compatible with modern  
recommendations to healthy dietary habits, and a method of  
feeding birds, in particular layers, that can be maintained  
15 under various rearing conditions (intensive, barn, plein  
air, free range, etc.) for the production of such eggs.

[0002] The significance of abbreviations used  
hereafter can be found in the part entitled "List of  
abbreviations".

20

Background of the invention and state of the art*Evolution of human diet*

[0003] For many years, food available from vegetable  
and animal sources was low in fat (less than 25% of total  
25 energy intake), for it was of a wild type and because it  
was mostly eaten raw or grilled. It was source of about  
equal amount of saturated and polyunsaturated fatty acids  
as well as of  $\omega 6$  and  $\omega 3$  isomers ( $P:S = \omega 6:\omega 3 = 1:1$ ).

[0004] When rudimentary agriculture began to slowly  
30 bring changes in dietary habits, making food from animal  
origin (meat, fish, milk, eggs) more present in the daily  
diet, the contribution of cholesterol and saturated fatty  
acids to the total fat content proportionally increased.

However, the  $\omega 6:\omega 3$  ratio remained relatively constant because traditional animal husbandry and fish culture did not widely differ in terms of feeding from former wild-type life, i.e. because the animals had large access to green-  
 5 leaf vegetables (livestock) and phytoplanktons (fish).

[0005] Deviations from human food standards came readily into prominence at the turn of the century with the emergence of the modern agriculture and vegetable-oil industry.

10 [0006] Emphasis on  $\omega 6$ -rich grains fattening of domestic livestock and fishery and on partial selective hydrogenation of  $\omega 3$ -rich vegetable oils results in a dramatic decrease in the amount of  $\omega 3$  fatty acids available to humans and to a not-less dramatic increase in  
 15 the contribution of total fat and *trans* fatty acids to the daily energy supply of the human diet.

[0007] The drastic deviation operated some 150 years ago in human food habits changed the  $\omega 6:\omega 3$  ratio that was about 1:1 during the evolutionary period to a now estimated  
 20 imbalance of about 10-11:1 for food from vegetable source and closer to 20-25:1 for mixed food from vegetable and animal sources. Meanwhile, the human genetic constitution remained relatively unchanged and did not cope with this brutal pace of change in the food chain.

25

Table 1

		Time (years)				
		-4.000.000	-10.000	1850	1950	2000
Total	fat	25%		30%	35%	40%
P:S		1:1		0.9:1	0.75:1	0.5:1
ω6:ω3		1:1			10:1	20:1

[0008] Table 1 gives a scheme of the relative contribution of different dietary fatty acids (saturated fatty acids,  $\omega 6$  and  $\omega 3$  polyunsaturated fatty acids) to the human diet and possible changes subsequent to modern  
5 agriculture and industrial food processing, involving fattening of animal husbandry and hydrogenation of fatty acids.

[0009] In the meantime, wild animals, which were still left grazing on green leaves and wild plant seeds,  
10 continued to exhibit balanced essential fatty acid ratio's in their fat depots, independently from the species they belong to (Crawford, M.A. et al. (1989) "The food chain for n-6 and n-3 fatty acids with special reference to animal products", in Dietary  $\omega 3$  and  $\omega 6$  Fatty Acids - Biological  
15 Effects . and Nutritional Essentiality (Galli, C. & Simopoulos, A.P., eds) NATO ASI Series A: Life Sciences Vol. 171, pp.5-19, Plenum Press, New York, ISBN 0-306-43231-5).

20 *Link with modern degenerative diseases*

[0010] Today,  $\omega 6$  and  $\omega 3$  PUFA are known to be essential in minimum amounts (see table 2 for adequate intake) for normal growth and development of humans. Their relative concentration or fractional ratio ( $\omega 6:\omega 3$ ) in food  
25 regulates the *in vivo* metabolism of lipoproteins, the fatty acid composition of cell membranes and the synthesis of some important biological mediators, the eicosanoids, which are essential to cell communication and global homeostasis. Scientific evidences suggest that at least some human  
30 degenerative diseases have a food link and that the highly unbalanced modern dietary  $\omega 6:\omega 3$  ratio, as well as the strong contribution of total fats (more than 35% of total energy intake) and of saturated fatty acids (P:S = 0.5:1,

including *trans* isomers) to the daily energy intake, may well have a direct implication into the appearance of some characteristic diseases of our modern society, i.e., cerebro- & cardiovascular diseases, coronary heart  
5 affections, cancers, diabetes, high blood pressure, chronic inflammatory and auto-immune diseases. More specifically, arachidonic acid, the  $\omega 6$  long-chain fatty acids derived from linoleic acid and/or obtained directly from food of animal origin, has been shown to effectively accumulate in  
10 modern man tissues when ingested in excess (A.T. Simopoulos (1991) Omega-3 fatty acids in health and disease and in growth and development, Am. J. Clin. Nutr. 54, 438-463). Consequently, arachidonic acid-derived eicosanoids have taken the lead in cell and tissue behaviour of modern man  
15 with all the deleterious consequences for health.

[0011] Scientific and epidemiological evidence seems to confirm that excess  $\omega 6$  fatty acids in modern man diet could well be one of the major culprit for reduced health performance in ageing.

20

*The "balanced diet" notion*

[0012] Galli & Simopoulos ("General recommendations on dietary fats for human consumption", in Dietary  $\omega 3$  and  $\omega 6$  Fatty Acids Biological Effects and Nutritional  
25 Essentiality (Galli, C. & Simopoulos, A.P., eds) pp. 403-404, North Atlantic Treaty Organisation Advanced Science Institute Series, Plenum Press, New York & London (1988)) defined a well-balanced diet as one that, among other things, provides max. 30% energy as fat together with a  
30 large proportion of monounsaturated fatty acids, an even distribution of saturated and polyunsaturated fatty acids (S:M:P = 1:6:1), a ratio between  $\omega 6$  and  $\omega 3$  isomers not exceeding 5 to 1 ( $\omega 6:\omega 3 \leq 5:1$ ), and an optimal amount of

350-400 mg  $\omega$ 3 long chain polyunsaturated fatty acids ( $\omega$ 3 LC-PUFA).

[0013] In a recent workshop held at The Cloisters, National Institute of Health (NIH) in Bethesda, Maryland, USA, April 7-9, 1999, an Expert Panel led by Prof. A.T. Simopoulos established the following adequate intakes for  $\omega$ 6 and  $\omega$ 3 fatty acids in adults (table 2).

Table 2: Adequate Intakes (AI) for Adults

Fatty Acid	Abbrev	Formula	Grams/day *	Cal/day	% total Cal.
Linoleic acid	LA	C18:2 $\omega$ 6	4.44	40	2
$\alpha$ -linolenic acid	LnA	C18:3 $\omega$ 3	2.22	20	1
EPA + DHA	$\omega$ 3 LCP	C20/22:5/6 $\omega$ 3	0.44	4	0.2

10

\*2000 kcal diet

[0014] It is seen that there is a clear trend towards re-adopting the historically-established, naturally-occurring lipid ratios settled by Nature 4 million years ago as those which must be optimally fit for human consumption. Quite remarkably, these modern recommendations for amounts and ratios of lipids in human diet are based on meta-analysis of epidemiological and clinical studies of the long term relationship between dietary habits and degenerative diseases in human.

20 [0015] Optimum dietary lipid pattern can be reached through substitution of  $\omega$ 6-rich for  $\omega$ 3-rich vegetable oils and enrichment of modern-type diets with greens, leaves and fish, but enrichment does in fine mean adding-up on fat.

[0016] Ideally, edible animal tissue lipids should also comply with these scientifically established evidence since pre-formed arachidonic acid from animal origin is known to incorporate very effectively in tissue lipids  
5 (Lands, W.E.M. (1997) Two faces of EFA, Inform 8, 1141-1147). Essentially, there is no scientific evidence for a need in dietary arachidonic acid in normal people. A very important aspect of wild-type animal tissue is that they favour  $\omega 3$  versus  $\omega 6$  long-chain phospholipids and contain  
10 substantially lower amount of arachidonic acid in comparison to their domesticated equivalent.

[0017] Scientifically and naturally established evidences suggest that healthy food from animal origins is of the wild or game-type, lean and balanced in essential  
15 fatty acids, low in arachidonic acid.

#### *Eggs as food*

[0018] Eggs are generally recognised as source of highly bio-available and valuable nutrients.

20 [0019] As a source of essential amino-acids, eggs are remarkable in that they are in perfect agreement with adult human needs; they are also a rich source of beneficial branched amino-acids (table 3).

Table 3: Essential amino-acids in eggs and human needs

Essential amino acids	(+)mg / 100 g (two 60-g eggs)	**RDA (mg) 70-kg adult
Histidine	275	-
*Isoleucine	675	840
*Leucine	1075	1120
Lysine	875	840
Cysteine + Methionine	712.5	700
Tyrosine + Phenylalanine	1162.5	1120
Threonine	587.5	560
Tryptophan	212.5	210
*Valine	825	980
<i>Lysine</i> <i>Cysteine + Methionine</i>	1.23	1.20

\*BCAA: branched chain amino-acids

\*\*RDA: recommended dietary allowances (National Academy of Sciences, U.S.A., 1974)

5 (+)FAO Food and Nutrition Paper 51(1990) Protein quality evaluation (Content in mg/g protein x 12.5)

[0020] Carbohydrates mainly appear in eggs as glucose and glycoproteins (table 4).

10

Table 4: Carbohydrates in eggs

	mg / 100 g (two 60-g eggs)		
	Albumen (68-g)	Yolk (32-g)	Total (100-g)
Free sugar*	260	260	520
Oligosaccharides**	330	110	440
Total	590	370	960

\*mainly D-glucose - \*\* N- & O-glycans in albumen, sialoglycans in yolk (Sugino, H., Nitoda, T. and Juneja, L.R. (1997) General Chemical Composition of Hen Eggs, in "Hen Eggs, Their Basic and Applied Science" (Yamamoto, T., Juneja, L.R., Hatta, H. & Kim, M. eds) CRC Press Inc., Ch. 2, pp. 13 - 24).

15

[0021] Lipids in egg yolk appear as a mixture of neutral and polar lipids; their fatty acids are either long (C16-18) or very long (C20-22) carbon chains (table 5).

5 Table 5: Lipids in eggs

Lipid fractions	g / 100 g (two 60-g eggs)			
	Triglycerides	Phospholipids	Cholesterol	Total
Weight contribution	6,9	2,7	0,430	10
% fatty acid	96	72	~0	85.5
Fatty acids type	C16-C18	C16-C22	-	C18

[0022] Vitamins and minerals are omnipresent at relatively high concentration in eggs (table 6).

**Table 6 : Vitamins, minerals and oligoelements in eggs and human needs**

Vitamins		Name	RDI*	100g-egg	%RDI
Vit.A	a	Retinol	1,5150	0,1921	12,7
*Vit.D	b	Calciferol	0,0100	0,0012	12,0
Vit.E	c	Alpha-tocopherol	20,0000	1,4000	7,0
*Vit.K1		Phylloquinone			
Vit.B1		Thiamin	1,5000	0,0620	4,1
Vit.B2		Riboflavin	1,7000	0,5080	29,9
*Vit.B3 (PP)	d	Niacin	20,0000	0,0740	0,4
*Vit.B5		Panhotenic acid	10,0000	1,2540	12,5
Vit.B6		Pyridoxin	2,0000	0,1400	7,0
Vit.B8 (H)		Biotin	0,3000	0,0200	6,7
Vit.B9 (M)		Folic acid	0,4000	0,0460	11,5
Vit.B12		Cyanocobalamine	0,0060	0,0010	16,7
Vit.C	e	Ascorbic acid	60,0000		

Minerals		Name	2000 Cal	100g-egg	%RDI
Ca		Calcium	1000	50,0	5,0
P		Phosphorus	1000	178,0	17,8
Mg		Magnesium	400	10,0	2,5
Na		Sodium		126,0	
Cl		Chlorine		174,2	
K		Potassium		120,0	
S		Sulfur		164,0	
Oligo		Name	2000 Cal	100g-egg	%RDI
Fe		Iron	18,00	1,440	8,0
Zn		Zinc	15,00	1,100	7,3
Se		Selenium			
Cu		Copper	2,00	0,014	0,7
I		Iodine	0,15	0,048	32,0
Mn		Manganese		0,024	
Mo		Molybden			

5 All numbers in mg; 100-g eggs stands for the edible part of two 60-g-eggs

RDI : Reference Daily Intake = average values for healthy Americans over 4-y-old

RDI\* given for a 2000 Cal-diet

10 \*: accessory- or conditionally essential nutrients

a) retinol equivalent : 1 mcg retinol/ $\beta$ -caroten or 3,3 IU

b) calciferol: 1 mcg or 40 IU

c) alpha-tocopherol equivalent ( $\alpha$ -TE) : 1 mg d-alpha-tocopherol or 1.49 IU

15 d) niacin equivalent : 1mg nicotinic acid (or nicotinamide) or 60 mg tryptophan

e) ascorbic or dehydroascorbic acid

[0023] The weight-distribution of amino-acids, carbohydrates and lipids is genetically encoded in eggs whilst that of vitamins, minerals and fatty acids is sensitive to their concentration in the bird's diet.

5 [0024] The egg lipid composition has often been criticised in terms of its relatively high concentration in saturated fatty acids and cholesterol compared to total energy content. The scientifically-proven relationship (known as the "lipid hypothesis") between high dietary CSI  
10 (cholesterol-saturated fat index) and relatively higher risk of cardio- and cerebrovascular diseases has often supported the critical evaluation of cholesterol content in food. Cell membranes of animal tissues differ from those of plant tissues in that they contain cholesterol and  
15 phospholipids bearing long chain polyunsaturated fatty acids. Egg yolk lipids conform to this rule: cholesterol and long-chain polyunsaturated fatty acids happen to appear in a ratio close to 1:1 (0.5 mM each in egg yolk) and the latter occur at the characteristic sn-2 position of tissue  
20 phospholipids. There is thus nothing special or "wrong" about the cholesterol level in egg - it just has to do with its primary function, i.e. to support the development of Life in avian. Cholesterol in egg yolk is almost a constant whatever the type of feed served to the chicken. However,  
25 the content of polyunsaturated fatty acids can be increased at the expenses of that of saturated and monounsaturated fatty acids by simple dietary means.

[0025] While amounts of saturated, mono-unsaturated,  $\omega$ 3 and  $\omega$ 6 polyunsaturated fatty acids have been separately  
30 varied in previous egg production and the effect of these minor changes on human blood lipid balanced were already described, eggs wherein all fatty acid and lipid fractions are controlled to result in a product which is compatible

with a healthy diet defined as that one designed by Nature in the wild, were never produced nor described.

#### Aims of the invention

5 [0026] The present invention aims to provide eggs obtained from domesticated birds, in particular layers, said eggs comprising an improved lipid balanced profile in order to result in a product compatible with healthy diet for humans and animals comparable to the one designed by  
10 nature in the wild.

[0027] Another aim of the present invention is to provide such safe and improved eggs and food compositions comprising such eggs that may be consumed as part of a balanced diet in reasonable quantity, as recommended by  
15 national and international heart foundations, and that sustain consumer's health in the long term.

[0028] A further aim of the present invention is to provide a feed composition of exclusive vegetarian origin and a feeding method for poultry, in particular layers,  
20 from which said eggs having an improved lipid balanced profile may be obtained.

[0029] A last aim of the present invention is to provide such feed composition and feeding method for feeding poultry, in particular layers, that can be  
25 maintained in various conditions for the production of such eggs.

#### Summary of the invention

[0030] The inventors have discovered that it is  
30 possible to obtain eggs from domesticated birds, preferably chicken eggs, having an advantageous lipid profile which complies with that of fat depots of wild- or game-type animals, that is balanced in saturated (30%) and polyunsaturated (30%) and is balanced in omega-6 (15%) and

omega-3 (15%) fatty acids. Said eggs will be hereafter called wild-type or game-type eggs.

[0031] More precisely, the eggs according to the invention has a lipid fraction balanced in seed and green  
5 plant-type  $\omega 6$  and  $\omega 3$  fatty acids according to the ratio of seed plant-type  $\omega 6$  fatty acids: green plant-type  $\omega 3$  fatty acids = 1:1  $\pm$  10%.

[0032] The eggs according to the invention is also characterised in that their lipid fraction is balanced  
10 between polyunsaturated and saturated fatty acids according to the ratio of polyunsaturated: saturated fatty acids = 1:1  $\pm$  10%.

[0033] The eggs according to the invention may be consumed or incorporated in food compositions in reasonable  
15 quantities as part of a balanced diet, these food compositions sustaining consumer health in the long term. They may be used as a functional food or as medicament (see for reference Milner J.A., *Journal of Nutrition* (1999), Volume 129, Number 7S, "Functional foods and health  
20 promotion", pages 1395S-1397S).

[0034] The phospholipid fraction in said eggs is also characterised by an advantageous balanced fraction of animal-derived long chain fatty acids: omega-6 ( $\omega 6$ ): omega-3 ( $\omega 3$ ) fatty acids equal to 1:3  $\pm$  10%.

25 [0035] Advantageously, the balanced lipid fraction of the eggs is made of green plant-type and animal-derived omega-3 ( $\omega 3$ ) fatty acids, characterised by the preferred following ratio, plant-type  $\omega 3$  fatty acids: animal-derived  $\omega 3$  fatty acids equal to 5:1  $\pm$  10%, a ratio similar to that  
30 recently proposed by the NIH Expert Panel for adequate intake of  $\omega 3$  fatty acids of plant and animal origins in human (table 2).

[0036] Preferably the eggs according to the invention have more than 450 mg/egg (about  $550 \pm 50$  mg/egg) of green plant-type fatty acids omega-3 ( $\omega 3$ ) and have more than 90 mg/egg (about  $110 \pm 10$  mg/egg) of animal-derived  
5  $\omega 3$  fatty acids.

[0037] Advantageously, the eggs according to the invention contain no more than about 40 mg/egg, preferably about  $35 \pm 5$  mg/egg, animal-derived omega-6 ( $\omega 6$ ) fatty acids and is essentially arachidonic acid.

10 [0038] Advantageously, the animal-derived omega-3 ( $\omega 3$ ) fatty acids contained in the eggs according to the invention are C20 and C22 fatty acids, preferably selected from the group consisting of eicosapentaenoic acid docosapentaenoic acid and docosahexaenoic acid.

15 [0039] Advantageously, said eggs contain about  $10 \pm 2$  mg eicosapentaenoic acid /egg, about  $15 \pm 3$  mg docosapentaenoic acid /egg and about  $75 \pm 15$  mg docosahexaenoic acid /egg.

[0040] Advantageously, the wild-type lipid profile  
20 of such eggs automatically results in an enrichment in vitamins, especially vitamin E with 20 mg/% of the edible part, and in a reduction of about 10% in their C12, 14, 16 and 18 saturated fatty acid content, with no more than 2.50g/% of the edible part, the cholesterol content of said  
25 eggs being no more than 375 mg/% of the edible part.

[0041] The wild-type lipid profile of such eggs results also in the accretion of a substantial amount of animal-derived  $\omega 3$  long chain polyunsaturated fatty acids (C20-C22) and in more than 50% reduction in arachidonic  
30 acid, comparatively to eggs available on the market.

[0042] Another aspect of the present invention is related to a feed composition of exclusive vegetarian origin in order to obtain from poultry, in particular

layers, such eggs, said composition being a wild-type diet containing no animal fats and satisfying the equation of Huyghebaert et al. (Arch. Geflügelk (1995) 59(2), p.145-152) exposed hereafter and comprising 4 to 10% (w:w) of  
5 total fat, seed  $\omega 6$  and green  $\omega 3$  plant-type fatty acids contributing to total fat content in steadily decreasing manner from respectively 40 to 15% and 50 to 30%, when total fat increases from 4 to 10%, the  $\omega 6$ :  $\omega 3$  essential fatty acid ratio being in favour of the green  $\omega 3$  plant  
10 type fatty acids and decreasing concomitantly from 0.8 to 0.5.

[0043] Preferably the feed composition according to the invention comprises about 30 to 40% carbohydrates, about 10 to 20% proteins, about 10 to 15% moisture, about 7  
15 to 12% ash and about 4 to 10% fats, the total being 100%, for a total metabolisable energy of about 2800 kcal. The total comprises also addition of vitamin A, vitamin D3, vitamin E, menadione sodium bisulfite, riboflavine, panthothenic acid, niacine, vitamin B6, folic acid, biotin,  
20 thiamin, vitamin B12 and oligoelements (Mn, Zn, Fe, Cu, I, Se, Co, Ca), the preferred composition being the one described in the table 9.

[0044] The present invention is also related to a feeding method of poultry, in particular layers, comprising  
25 the step of feeding poultry with the feed composition according to the invention in order to readjust the  $\omega 6$ : $\omega 3$  ratio in eggs, so that they present an improved balanced fatty acids composition according to the invention.

[0045] A last aspect of the present invention is  
30 related to a food composition comprising, as a food ingredient, the whole egg, the egg white or the egg yolk of the eggs according to the invention, especially a food

composition suitable for human consumption, including a functional food.

[0046] The present invention will be described in more details in the following detailed description of the invention and in the following examples.

#### Detailed description of the invention

##### Designing the feed

[0047] The best feeding option would be the natural, wild-type one, whereupon the bird grazes on greens and leaves and collects insects and worms in a wild, "fight-or-flight" environment.

[0048] However, this method of production is not compatible with the growing need of an ever-expanding world population and economy. Greens are essentially source of  $\alpha$ -linolenic acid that is formed from linoleic acid in plant chloroplasts as a result of energy transfer from sunlight to chemical  $\pi$ -bonds. Given that the lipid fraction of green leaves account for about 1% of their total mass and that about 50% fatty acids therein are  $\alpha$ -linolenic acid, one can calculate that a normal 100-g portion leaves provides 0.5 g  $\alpha$ -linolenic acid. This is far from negligible when compared to the low concentration of this particular fatty acids in most edible seeds and oils, but total fat content ( $\pm 1\%$ ) is much too low to sustain a continuous process of egg production (an economically-viable way to produce healthy eggs at reasonable cost on a world-wide basis).

[0049] Within the group of green lipids, fits a unique exception from seeds in the name of flax seeds. Flax or linseeds are indeed an exceptional source of plant  $\omega 3$  fatty acids otherwise found in much smaller amounts and higher  $\omega 6:\omega 3$  ratio in soya and canola seeds (table 7). Flaxseeds are also readily available at competitive world

market price for feed ingredients. In this sense, flax seeds represent a unique source of green plant-type lipids which is contained within a seed in substantial quantities and at reasonable cost.

5

Table 7.  $\omega$ 3-containing greens, flaxseeds and Designer Feed  
(% of triglycerides)

Vegetable plant	SAFA	MUFA	PUFA		
	-	$\omega$ 7 + $\omega$ 9	$\omega$ 6	$\omega$ 3	$\omega$ 6: $\omega$ 3
Cabbage, red	25	5	30	40	0.75
Designer Feed	12	18	25	45	0.58
Parsley	18	3	26	54	0.48
Lettuce	18	3	17	44	0.38
Cabbage, white	18	8	15	58	0.26
Flax seeds	9	18	15	57	0.26
Cauliflower	22	15	13	50	0.26
Brussels sprouts	20	5	12	63	0.19
Spinach	12	3	8	52	0.16

- 10 [0050] Huygebaert (Arch. Geflügelk (1995) 59(2), p.145-152) has developed a mathematical model for the prediction of the fat composition in egg, in particular C16-C18, based on their respective contribution to total fat in the feed (table 8).

Table 8. Response in the egg yolk fat (%)

y	Intercept	x <sub>1</sub>	x <sub>2</sub>	(x <sub>1</sub> ) <sup>2</sup>	(x <sub>2</sub> ) <sup>2</sup>	x <sub>1</sub> x <sub>2</sub>
C16:0	26.60	-1.462	0.191	0.0348	-0.0046	0.028
C18:0	7.94	-0.178	-0.121	0.0069	0.0029	0.010
C18:1	41.70	-2.637	0.378	0.0464	-0.0005	0.042
C18:2	-9.26	2.559	0.322	-0.1311	-0.0011	0.026
C18:3	-0.03	0.311	-0.016	-0.0202	0.0031	0.023

x<sub>1</sub>: dietary fat level-%; x<sub>2</sub>: the level-% of the respective characteristic in the dietary fat.

5 [0051] Therefore, this models predicts for example that a concentration y of C16:0 fatty acids in the eggs can be obtained if the diet given to the poultry contains a concentration x<sub>1</sub> of dietary fat and a concentration x<sub>2</sub> of C16:0 fatty acids, the concentration y being calculated as  
10 follows:

$$y = 26.60 - 1.462x_1 + 0.191 x_2 + 0.0348(x_1)^2 - 0.0046(x_2)^2 + 0.028x_1x_2$$

[0052] This model has been shown to be valid for the  
15 continuous production of wild-type eggs as defined, if the feed composition given to poultry comprises 4 to 10% (w:w) of total fat, seed ω6 and green ω3 plant-type fatty acids contributing to total fat content in steadily decreasing manner from 40 to 15% and from 50 to 30%, respectively, and  
20 ω6:ω3 essential fatty acid ratio being in favour of the green ω3 plant type fatty acids and decreasing from 0.8 to 0.5. More precisely, the fat composition (ω6:ω3 = Polyunsaturated:Saturated = 1:1) of the wild-type egg has been maintained constant for several months (> 18 months)  
25 under a defined feeding regimen.

[0053] Groups of 30,000 Isabrown birds were fed with a wild-type diet containing no animal fat, 35.5% carbohydrates, 17% protein, 12% moisture, 10.25% ash and

6.5% fat for a total of 2,800 Kcal metabolisable energy (M.E.). The detailed composition of the feed is given in Table 9.

5 Table 9. Composition of Designed Feed

Raw Material Inclusion	(%)	Analysis	(%)	
Avizym 2300 - 20%	0.50	Protein	17.0	
Choline - 75%	0.05	Fat	6.5	
Limestone gran.	8.29	Carbohydrate	35.5	
Layer supplement	0.25	Moisture	12.0	
Salt	0.20	Ash	10.25	
Dical. Phos.	1.26	M.E.	2,800 Kcal	
D,L-methionine - 40%	0.28			
Course wheat	47.5		Total	Digestible
Course peas	10.0		(%)	(%)
Wheat middlings	2.785	Ca	3.7	-
Soya 50 / Hypro	15.33	P	0.58	0.32
Sunflower meal 30	6.0	Lys	0.89	0.74
profat		Met	0.38	0.34
Lucern 20 <sup>®</sup> (350)	2.53	Cys+Met	0.68	0.57
		Thr	0.61	0.49
Subtotal (DSF)	95.0	Trp	0.20	0.165
		Lys/(Cys+Met)		1.30
Vegetable oil (DM)	5.0			
Total	100			

[0054] Layer supplement provides the following per kilogram of diet: vitamin A, 10,000 I.U.; vitamin D3,

2,000 I.U.; vitamin E, 10 I.U.; menadione sodium bisulfite, 0.6 mg; riboflavin, 5 mg; pantothenic acid, 10.9 mg; niacin, 40 mg; vitamin B6, 1 mg; folic acid, 0.5 mg; biotin, 20 µg; thiamine, 1 mg; vitamin B12, 20 µg; Mn, 5 75 mg; Zn, 55 mg; Fe, 35 mg; Cu, 7.5 mg; I, 1.9 mg; Se, 0.1 mg; Co, 0.7 mg, Ca, 330 mg; Mg, 55 mg.

[0055] Vegetable oil is cold-pressed flax seed oil (BS 6900: sediment, max. 0.25%; Iodine Value, min. 175; Acid Value, max. 4 mg KOH/g, Peroxide Value, max. 10, 10 Colour Gardner, max. 13) stabilised with 0.4% Rendox (Kemin) containing BHA (E320), Ethoxyquin (E324), Citric Acid (E330), phosphoric acid (E338), mono- & diglycerides of fatty acids (E471), and enriched with 0.2% dl- $\alpha$ -tocopherol (Roche).

15

#### Designing the wild-type egg

[0056] The composition of fatty acids in egg yolk lipids can be modulated through dietary means. Especially, the  $\omega$ 3 fatty acid of plant origin ( $\alpha$ -linolenic acid) can 20 be incorporated at the expenses of saturated and monounsaturated fatty acids in the triglyceride fraction. Typically, regular eggs would contain almost undetectable level (< 1%) of  $\alpha$ -linolenic acid whilst free wandering birds grazing on greens and worms would almost incorporate 25 13% of  $\alpha$ -linolenic and show a balanced  $\omega$ 6: $\omega$ 3 ratio in the triglyceride fraction. Nutritionally, this change into the birds diet does not affect the way these lipids are metabolised in the body since  $\alpha$ -linolenic acid is usually burned and incorporated into tissues and cells membrane 30 lipids at the same rate as monounsaturated fatty acids. A clear sign that this is indeed so is the fact that most  $\alpha$ -linolenic acid, when present in the yolk triglyceride fraction, is located at position sn-1/3, characteristic of

non-essential fatty acids. However, it provides a unique back-up source of  $\omega$ 3 fatty acids in fat depots for the synthesis of DHA through the fatty acids cascade pathway.

[0057] The major change associated with the presence  
5 of  $\alpha$ -linolenic in the wild birds diet is the ratio inversion of the animal derived long-chain polyunsaturated fatty acids in the phospholipid fraction of the yolk. Whilst regular feed contributes to the accretion of arachidonic acid at the expenses of docosahexaenoic acid  
10 (AA:DHA = 2:1), the wild-type feed favours the synthesis and deposition of docosahexaenoic acid (AA:DHA = 1:3).

Characteristics of wild-type eggs obtained with the designed feed

15 *Fatty acid and lipid compositions of wild-type eggs compared to those of standard eggs*

[0058] After 3 weeks induction on Designer Feed, wild-type eggs can be followed for their fatty acid pattern. A validation study ran on five groups of hens  
20 during 19 months has allowed to establish a specification for the wild-type egg (table 10), as follows:

Table 10. Fatty acid and lipid composition of wild-type eggs

Fatty acids	% rel.	Lipids	% rel.
C16:0	19.34 ± 0.71	Σ(SAFA)	28.5 ± 1.1
C18:0	9.18 ± 0.88	Σ(MUFA)	40.9 ± 1.7
C16:1ω7	3.17 ± 0.42	Σ(PUFA)	28.7 ± 1.6
C18:1ω9	37.74 ± 1.45	P/S	1.00 ± 0.08
C18:2ω6	13.59 ± 0.76	ω6:ω3	1.01 ± 0.07
C18:3ω3	11.69 ± 1.26	ω6:ω3 LCP	0.32 ± 0.03
C20:4ω6	0.81 ± 0.14	*C20:4ω6	35 ± 5 mg
C20:5ω3	0.28 ± 0.06		
C22:5ω3	0.43 ± 0.10		
C22:6ω3	1.86 ± 0.39		

\*Content of arachidonic acid (AA, C20:4ω6) calculated as %rel. x 4,200 mg fatty acids per 50-g egg edible portion.

5

[0059] During the same period, standard eggs available on the market and obtained from Europe, the United States, South and East Asia were collected and analysed according to the same procedure for their fatty acid and lipid contents. Results show that standard eggs substantially deviate from wild-type egg (table 11). In particular, standard eggs are extremely poor (max. 1%) in plant-type ω3 fatty acids and, as a consequence, they are also much richer in arachidonic acid.

15

Table 11. Fatty acid and lipid composition of standard eggs

Fatty acids	% rel.	Lipids	% rel.
C16:0	22.63 ± 2.32	Σ(SAFA)	31.0 ± 2.5
C18:0	8.37 ± 0.75	Σ(MUFA)	44.8 ± 3.7
C16:1ω7	3.26 ± 0.89	Σ(PUFA)	20.9 ± 4.6
C18:1ω9	41.5 ± 3.30	P/S	0.68 ± 0.18
C18:2ω6	17.03 ± 4.06	ω6:ω3	12.03 ± 5.0
C18:3ω3	0.66 ± 0.37	ω6:ω3 LCP	2.06 ± 1.0
C20:4ω6	2.03 ± 0.30		
C20:5ω3	0.01 - 0.02		
C22:5ω3	0.13 ± 0.05		
C22:6ω3	1.03 ± 0.40	*C20:4ω6	85 ± 15

\*Content of arachidonic acid (AA, C20:4ω6) calculated as %rel. x 4,200 mg fatty acids per 50-g egg edible portion.

5 [0060] Other eggs, often declared as ω3 docosahexaenoic acid enriched, were analysed in detail for their fatty acids distribution. These eggs are normally obtained from hens fed with a docosahexaenoic acid oil-enriched feed (fish oil, algae, single cell oil, etc.) and

10 their content of this specific fatty acid is relatively higher than in other eggs. It also turned out that such eggs exhibit a fatty acid composition reminiscent to that of standard eggs in terms of their low content in wild-type plant ω3 fatty acids (max. 1%) and their high content in

15 domestic-type animal-derived ω6 long-chain fatty acids, not withstanding the additional fact that, when animal-derived ω3 fatty acids were summed up, they had less of these than the wild-type egg obtained with an exclusive vegetarian diet.

*Stability of wild-type eggs compared to that of standard eggs*

[0061] Wild-type eggs, naturally rich in  $\omega 3$  fatty acids, may be assumed less stable than modern  $\omega 6$ -rich eggs. Nutritionists and consumers may raise more specific concerns towards fatty acids and cholesterol peroxidation in wild-type eggs.

*a. Stability with hen's age*

10 [0062] Fatty acid changes with hen's age. The influence of hen's age on  $\omega 3$  long-chain phospholipids content in wild-type eggs was followed on the life-cycle of two independent groups of 30,000 birds each. The data show that although a very slight trend down is observed, the effect of age on fatty acid composition in wild-type eggs is minor (table 12).

Table 12. Content of  $\omega 3$  long-chain phospholipids in wild-type egg as a function of hen's age

Group 1		Group 2	
Week	$\Sigma \omega 3$ LCP (%)	Week	$\Sigma \omega 3$ LCP (%)
38	2.62	26	2.59
43	2.49	31	2.77
46	2.62	34	2.75
49	2.53	44	2.35
52	2.45	48	2.41
56	2.31	56	2.67
60	2.28	61	2.45
70	2.53	$m \pm \sigma$	$2.57 \pm 0.15$
$m \pm \sigma$	$2.48 \pm 0.15$		

20  $\Sigma \omega 3$  LCP (%) as sum of EPA + DPA + DHA

*b. Stability with egg's age*

[0063] Fatty acid changes with egg's age. Wild-type eggs were analysed for their fatty acid composition (from Fatty Acid Methyl Ester (FAME) spectrum analysis) 3 and 9 weeks after being laid and stored at room temperature (21°C). After 9 weeks, the yolk were barely separable from the white. The most obvious change upon storage is in the level of docosahexaenoic acids (-9% / 3 weeks, -18% / 6 weeks and -27% / 9 weeks at 21°C). All other fatty acids were kept at a remarkable constant level (table 13).

Table 13. Changes in fatty acid composition of wild-type egg with age

Fatty acids	% rel.		
	Fresh	3-w	9-w
C16:0	18.48	18.72	18.50
C18:0	8.72	8.16	8.45
C16:1 $\omega$ 7	3.29	3.58	3.38
C18:1 $\omega$ 9	39.96	40.05	39.66
C18:2 $\omega$ 6	13.17	13.60	14.19
C18:3 $\omega$ 3	11.00	11.10	11.14
C20:4 $\omega$ 6	0.74	0.77	0.79
C20:5 $\omega$ 3	0.25	0.22	0.24
C22:5 $\omega$ 3	0.42	0.31	0.33
C22:6 $\omega$ 3	1.86	1.69	1.36

Lipids	% rel.		
	Fresh	3-w	9-w
$\Sigma$ (SAFA)	27.2	26.9	26.9
$\Sigma$ (MUFA)	43.2	43.6	43.0
$\Sigma$ (PUFA)	27.4	27.7	28.0
P/S	1.01	1.03	1.04
$\omega$ 6: $\omega$ 3	1.03	1.08	1.15
$\omega$ 6: $\omega$ 3 LCP	0.29	0.35	0.41
*C20:4 $\omega$ 6	31.1	32.3	33.2

15 \*Content of arachidonic acid (AA, C20:4 $\omega$ 6) calculated as %rel. x 4,200 mg fatty acids per 50-g egg edible portion. Ref. Anal. Malvoz 97-05-05

*c. Stability with processing temperatures*

[0064] Fatty acid changes with processing temperatures. Both wild-type and standard eggs were tested for their stability against oxidation during typical

culinary practices, i.e., boiling (hard eggs) and baking (cake and baked custard) (tables 14 & 15) and compared.

5 Table 14. Changes in fatty acid composition of wild-type egg upon cooking

Fatty acids	% rel.			
	Fresh	Boiled	Cake	Custard
C16:0	18.48	18.34	17.97	18.81
C18:0	8.72	8.49	8.48	8.78
C16:1 $\omega$ 7	3.29	3.17	2.00	3.04
C18:1 $\omega$ 9	39.96	37.9	37.00	37.68
C18:2 $\omega$ 6	13.17	14.1	16.56	14.68
C18:3 $\omega$ 3	11.00	12.64	12.20	11.48
C20:4 $\omega$ 6	0.74	0.74	0.71	0.71
C20:5 $\omega$ 3	0.25	0.26	0.21	0.18
C22:5 $\omega$ 3	0.42	0.44	0.42	0.42
C22:6 $\omega$ 3	1.86	1.74	1.76	1.83

Table 15. Changes in fatty acid composition of standard egg upon cooking

Fatty acids	% rel.			
	Fresh	Boiled	Cake	Custard
C16:0	22.51	-	22.64	22.97
C18:0	8.11	-	7.63	8.57
C16:1 $\omega$ 7	3.46	-	3.27	3.52
C18:1 $\omega$ 9	40.32	-	40.13	41.74
C18:2 $\omega$ 6	17.84	-	19.87	15.74
C18:3 $\omega$ 3	0.79	-	1.48	0.69
C20:4 $\omega$ 6	2.18	-	1.80	1.90
C20:5 $\omega$ 3	0.01	-	0.03	0.02
C22:5 $\omega$ 3	0.17	-	0.13	0.13
C22:6 $\omega$ 3	1.16	-	1.02	0.97

- 5 [0065] These results show that the fatty acid composition of fresh and cooked wild-type and standard-type eggs are identical within the limit of accuracy.

*d. Setting upon boiling*

- 10 [0066] The rates of setting of the eggs upon boiling were also compared between standard and wild-type eggs. Eggs were bored in order to have a hole in the shell at the apical side where the air chamber lies (without perforating it) and were boiled for various time (8 to 12 min.) in hot  
 15 water (min. 400 ml per egg). At the end of the incubation, eggs were rapidly cooled in a large volume of cold water and stored one night in the fridge. The day after, the eggs were peeled and sliced in twice. The appearance of the yolk in standard and wild-type eggs was compared. No difference

in rate of setting could be assigned at any time between the two types of eggs.

*Cholesterol content in wild-type eggs compared to standard*  
5 *eggs*

[0067] Wild-type and standard eggs were boiled, cooled, dried and peeled. The white was separated from the yolk and the cholesterol content in the yolk was determined. Based on the weight of the different fractions  
10 (intact egg, shell & membranes, albumen, yolk), the amount of cholesterol in 100-g egg edible portion of wild-type and standard eggs was calculated (table 16).

Table 16. Cholesterol in wild-type and standard eggs

	Standard eggs(a)	Wild-type eggs(b)
Total weight (g)	63.0 ± 7.0	65.8 ± 5.4
Shell & membranes (g)	7.0 ± 0.5	7.3 ± 0.8
Albumen (g)	39.3 ± 2.70	40.2 ± 4.3
Yolk (g)	17.8 ± 1.9	18.2 ± 2.0
Yolk (g/% edible)	31.2 ± 3.2	31.3 ± 2.9
Cholesterol/yolk (%)	216 ± 24	217.8 ± 28
Cholesterol (mg/g yolk)	12.2 ± 1.0	12.0 ± 1.0
Cholesterol/egg (mg/% edible)	380 ± 45	375 ± 45

15 (a) 26 analyses, (b) 45 analyses - all confirmed by two independent laboratories.

[0068] The cholesterol content of both standard eggs and wild-type eggs is around 375 ± 45 mg/%, of the edible part. In other words, the cholesterol of the wild-type eggs

according to the invention is not changed as compared to the one of standard-type eggs.

### 5 Vitamin E content in wild-type eggs compared to standard eggs

[0069] Among the antioxidants present in eggs, vitamin E plays an essential role in stabilising the lipids against oxidation and rancidity. Wild-type eggs, richer in sensitive  $\omega 3$  fatty acids, are advantageously enriched with  
 10 vitamin E in order to avoid peroxidation of cholesterol and other lipid fractions. Designer feed is enriched with 0.2% dl- $\alpha$ -tocopherol acetate in order to maintain 10 mg vitamin E per 50-g edible egg (table 17).

15 Table 17. Vitamin E content (mg/%) in wild-type eggs compared to standard-type eggs

	Standard	Wild-type				
Egg tested	Egg N°1	Egg N°2	Egg N°3	Egg N°4	Egg N°5	Average *
$\alpha$ -toco pherol	6.2	18.7	23.9	19.8	19.3	20.4 $\pm$ 0.2
$\gamma$ -toco pherol	2.1	2.03	1.9	2.15	2.3	2.1 $\pm$ 0.2

\*(mg/%) a 50-g egg edible portion contains min. 10 mg vitamin E.

### 20 Lysozyme content in wild-type eggs compared to standard eggs

[0070] The effect of changing dietary lipids on hen's capacity to produce essential active enzymes and proteins for the protection of eggs against invasion by  
 25 pathogens was tested by measuring the amount of lysozyme

present in wild-type egg as compared to that in standard eggs (table 18).

Table 18. Lysozyme content in Standard and Wild-type eggs

Lysozyme	<i>Standard type</i>	Wild type
mg/g dry matter albumen	32.4 $\pm$ 1.3	33.5 $\pm$ 1.5

5

21 analyses on each type egg, from layers of 42 to 65 weeks of age for standard eggs and from layers of 32 to 55 weeks of age for wild-type eggs.

- 10 [0071] As seen, the amount of lysozyme contained in the wild-type egg is similar to the one contained in standard eggs.

Nutritional make-up of the wild-type egg to human

- 15 [0072] Egg lipids are made of fat store lipids (triglycerides, TG) and structural lipids (phospholipids, PL and cholesterol, CHL). These occur in egg yolk in a constant specific ratio (TG:PL:CHL = 16:6:1). Most fatty acids are concentrated in the triglyceride and the
- 20 phospholipid fractions whilst cholesterol is almost totally (90-95%) unesterified. Fatty acids in the two fractions are not randomly distributed: essential fatty acids are mostly present at position sn-2 of the triglyceride and the phospholipid fractions whilst non-essential fatty acids
- 25 (FA) occur at position sn-1/3 of the triglyceride fraction and at position sn-1 of the phospholipid fraction.

- [0073] The distribution of fatty acids in egg yolk lipids drives their postprandial influence on blood lipids. In the digestive tract, they are hydrolysed by pancreatic
- 30 1,3-lipase and 2-phospholipase to free fatty acids, sn-1,3 monoglycerides and sn-2 lysophospholipids, respectively.

Their reconstitution in the intestinal enterocytes results into the formation of triglycerides bearing essential fatty acids at position sn-2 and, among others, long-chain polyunsaturated fatty acids at position sn-1,3.

5 Triglycerides with essential fatty acids at position sn-2 are known to have hypocholesterolaemic effect in human whilst long-chain polyunsaturated fatty acids at position sn-1,3 of blood triglycerides are directly available for tissue incorporation through their release by endothelial

10 1,3-lipase. The bioavailability of egg yolk long-chain polyunsaturated fatty acids is similar to that of those from other animal tissues and must be very similar to that of endogenously produced long-chain polyunsaturated fatty acids.

15

#### Prophylactic effects of egg lipids

[0074] Given that egg yolk long-chain polyunsaturated fatty acids are extremely bio-available for incorporation into tissue- and circulating cells membrane

20 lipids and that their  $\omega 6:\omega 3$  ratio is susceptible to changes through dietary means, it is interesting to assess the influence of the bird's diet on the healthiness of the eggs destined to human consumption.

[0075] Egg lipids contain small amounts (less than

25 20% of total fatty acids content) of short and medium chain (C12-16) fatty acids. Their location at position sn-1/3 of TG and sn-1 of PL makes them available for direct energy production or for storage in adipose tissue. Monounsaturated fatty acids and polyunsaturated fatty acids

30 present at position sn-2 of triglycerides contribute to the hypocholesterolaemic effect of egg lipids. Long-chain polyunsaturated fatty acids located at position sn-2 of PL's are available for tissue incorporation.

[0076] Seen as a food lipid vector, egg is ranking high in the range of dietary fats (dairy and meat produces, vegetable and fish oils). Fish oil is often reckoned as a good source of  $\omega$ 3 long-chain polyunsaturated fatty acids. However, two-third of long-chain polyunsaturated fatty acids in fish oil are associated with the sn-2 position of the TG which makes them less bio-available and more susceptible to be diluted in fat depots and thus more prone to oxidative deterioration. It has been recommended to take large amount of vitamin E supplements together with fish oil.

[0077] As compared to regular eggs that are almost totally depleted of the wild-type plant linolenic acid (linolenic acid (LnA) < 1%; ratio linoleic acid:  $\alpha$ -lineolenic acid (LA:LnA) > 30:1), wild-type eggs supply these two essential fatty acids in a balanced ratio (LA:LnA = 1:1) and contribute to the endogenous synthesis of long-chain polyunsaturated fatty acids via the fatty acids biological cascade in the liver. The absence of  $\alpha$ -linolenic acid in the regular diet of layers also leads to the preferential accretion of arachidonic acid in egg yolk phospholipids ( $\omega$ 6: $\omega$ 3 LC-PUFA = 2:1) whilst this ratio is inverted in wild-type eggs ( $\omega$ 6: $\omega$ 3 LC-PUFA = 1:3). Direct incorporation of dietary long-chain polyunsaturated fatty acids in tissues and circulating cells is thus in favour of  $\omega$ 3 long-chain polyunsaturated fatty acids with the wild-type eggs.

[0078] It is known that the effect of  $\omega$ 3 fatty acids on serum cholesterol concentration is similar to those of other unsaturated fatty acids (monounsaturated and  $\omega$ 6 polyunsaturated), i.e., when they replace C12-16 saturated fatty acids at the sn-2 position of triglycerides, they

lower serum cholesterol.  $\omega$ 3 long-chain polyunsaturated fatty acids have an added benefit of consistently lowering serum triglyceride concentration through reduction of chylomicron and VLDL secretion by the intestine and the  
5 liver, respectively.

[0079] When fed to selected groups of people, the eggs according to the invention have been shown to indeed contribute to the improvement of: (a) circulating cell membranes fatty acid composition ( $\omega$ 3: $\omega$ 6 long-chain  
10 polyunsaturated fatty acids ratio), (b) blood lipid distribution (no statistical change in blood cholesterol level, improved distribution within blood lipoproteins - HDL/LDL balance, substantial reduction in the amount of fat circulating in the blood), (c) blood pressure (5 to 10%  
15 reduction in both systolic and diastolic pressure) and, even (d) breast milk lipid composition (60% and 300% increase in  $\alpha$ -linolenic acid and docosahexaenoic acid, respectively, with no substantial changes in other fatty acids).

20 [0080] Finally, through this feeding practice of the chicken, it has been feasible to readjust the  $\omega$ 6: $\omega$ 3 ratio in eggs so that they present a balanced fatty acid composition comparable to the original "wild-type food" available to early man. As a lipid source, the egg  
25 according to the invention thus belongs to the minor family of  $\omega$ 3-rich fats and oils and lies in between those from vegetable and fresh water fish origins (Table 19).

Table 19.  $\omega$ 3-containing seeds, fish oils compared to wild-type egg

(% of triglycerides)

Vegetable/fish lipid source	SAFA	MUFA	PUFA		
	-	$\omega$ 7 + $\omega$ 9	$\omega$ 6	$\omega$ 3	$\omega$ 6: $\omega$ 3
Wheat germ	20	18	55	7	8
Soybean	16	22	54	7.5	7
Walnut	11	15	62	12	5
Canola	7	63	20	10	2
Egg according to the invention	30	40	13	13	1
Salmon	20	30	5	5	1
Trout	25	30	6	6	1

5

[0081] The eggs according to the invention and river fish deliver a minimum of 70% unsaturated fatty acids (the healthy one), equal amounts of both  $\omega$ 6 and  $\omega$ 3 polyunsaturated fatty acids ( $\omega$ 6: $\omega$ 3 = 1:1) and substantial  
 10 amounts of animal-derived  $\omega$ 3 long-chain polyunsaturated fatty acids in a favourable ratio ( $\omega$ 6: $\omega$ 3 = 1:3) (table 20).

Table 20.  $\omega$ 3 LC-PUFA in the egg according to the invention and river fish

15

$\omega$ 6: $\omega$ 3	PUFA	LC-PUFA
Egg according to the invention	1.03	0.35
Salmon	0.98	0.32
Trout	0.92	0.20

[0082] Furthermore, the eggs according to the invention are also characterised by advantageous organoleptic properties in terms of freshness and flavour. They are rich in vitamins and antioxidants and are produced  
5 from layers effectively maintained immunised against Salmonella infections through the diet according to the invention they receive, said diet being rich in oligosaccharides naturally present in green and leaves.

LIST OF ABBREVIATIONS

- P: polyunsaturated fatty acid
- 5 S: saturated fatty acid
- M: monounsaturated fatty acid
- AA: arachidonic acid
- EFA: essential fatty acid
- FA: fatty acid
- 10 LC-MUFA: long-chain monounsaturated fatty acid
- LC-PUFA: long-chain polyunsaturated fatty acid
- LCP: long-chain polyunsaturated fatty acid
- PUFA: polyunsaturated fatty acid
- MUFA: monounsaturated fatty acid
- 15 SAFA: saturated fatty acid
- EPA: eicosapentaenoic acid
- DHA: docosahexaenoic acid
- DPA: docosapentaenoic acid
- LnA:  $\alpha$ -linolenic acid
- 20 LA: linoleic acid
- CHL: cholesterol
- CSI: cholesterol-saturated fat index
- TG: triglyceride
- PL: phospholipid
- 25 VLDL: very low density lipoprotein
- HDL: high density lipoprotein
- LDL: low density lipoprotein
- $\omega$ 3 fatty acids: fatty acids having first unsaturation on carbon 3 from the terminal methyl, as known by the man
- 30 skilled in the art
- $\omega$ 6 fatty acids: fatty acids having first unsaturation on carbon 6 from the terminal methyl, as known by the man skilled in the art

CLAIMS

1. An egg obtained from a domesticated bird, in particular a layer, having a lipid fraction balanced in seed and green plant-type  $\omega 6$  and  $\omega 3$  fatty acids according to the ratio of seeds plant-type  $\omega 6$  fatty acids / green plant-type  $\omega 3$  fatty acids =  $1:1 \pm 10\%$  and having a lipid fraction balanced between polyunsaturated and saturated fatty acids according to the ratio of polyunsaturated / saturated fatty acids =  $1:1 \pm 10\%$ .
2. The egg according to claim 1, characterised in that its phospholipid fraction is balanced according to the ratio of animal-derived  $\omega 6$  fatty acids / animal-derived  $\omega 3$  fatty acids =  $1:3 \pm 10\%$ .
3. The egg according to claim 1 or claim 2, balanced in plant-type and animal-derived  $\omega 3$  fatty acids according to the ratio of green plant-type  $\omega 3$  fatty acids / animal  $\omega 3$  fatty acids =  $5:1 \pm 10\%$ .
4. An egg according to claim 1, comprising green plant-type fatty acids ( $\omega 3$ ) in a concentration comprised between 450 and 600 mg/egg.
5. The egg according to any one of the preceding claims, whose animal-derived  $\omega 3$  fatty acids account for more than 90 mg/egg and whose composition comprises C20 & C22  $\omega 3$  fatty acids, the C20 and C22 fatty acids being preferably selected from the group consisting of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid.
6. The egg according to any one of the preceding claims, which contains no more than 40 mg/egg of animal-derived  $\omega 6$  fatty acids, and which is essentially arachidonic acid.

7. The egg according to any one of the preceding claims, which contains no more than 2.5 g saturated fatty acids and 375 mg cholesterol /% of the edible part.

5                   8. Feed composition of exclusive vegetarian origin for poultry, in particular layers, able to produce the egg according to any one of the preceding claims, characterised in that it satisfies the mathematical model of Huygebaert (Huygebaert (Arch. Geflügelk (1995) 59(2),  
10 p.145-152)), said composition containing no animal fat and comprising as ingredients 4 to 10% (w:w) of total fat, seed  $\omega 6$  and green  $\omega 3$  plant-type fatty acids contributing to total fat content in steadily decreasing manner, 40 to 15% (w:w) and from 50 to 30% (w:w), respectively, and  $\omega 6:\omega 3$   
15 essential fatty acid ratio being in favour of the green  $\omega 3$  plant type fatty acids and decreasing from 0.8 to 0.5, the total (w:w) of the ingredients of the composition being 100%.

9. Method for obtaining the egg according to  
20 any of the claims 1 to 7 from poultry, comprising the step of feeding said poultry with the composition according to claim 8.

10. A food composition comprising, as a food ingredient, the whole egg, the egg white or the egg  
25 yolk of the egg according to any one of the claims 1 to 7, in particular a food composition suitable for human consumption.

## INTERNATIONAL SEARCH REPORT

Interr. Application No

PCT/BE 01/00084

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23L1/32 A23L1/30 A23K1/18 A23K1/14

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EDER K.: "Laying performance and fatty acid composition of egg yolk lipids of hens fed diets with various amounts of ground or whole flaxseed" ARCHIV FÜR GEFLÜGELKUNDE, vol. 62, no. 5, 1998, pages 223-238, XP000956233 the whole document	1-10
X	DE 43 14 899 A (WOOBANG LAND CO) 9 December 1993 (1993-12-09) page 2, line 3 -page 4, line 53; claims 1-8,10 -/-	1-10

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## ° Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

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\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

3 September 2001

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Boddaert, P

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	<p>AN B.: "Effects of dietary fat sources containing omega3 or omega6 polyunsaturated fatty acids on fatty acid composition of egg yolk in laying hens"</p> <p>KOREAN JOURNAL OF ANIMAL SCIENCE, vol. 41, no. 3, 1999, pages 293-310, XP000956227 the whole document</p>	1-10
A	<p>VAN ELSWYCK M.: "Nutritional and physiological effects of flax seed in diets for laying fowl"</p> <p>WORLD'S POULTRY SCIENCE JOURNAL, vol. 53, no. 3, 1997, pages 253-305, XP000956232 the whole document</p>	1-10
A	<p>DANICKE S.: "Influence of graded levels of rape seed in laying hen diets on the fatty acid composition of the yolk fat"</p> <p>FETT WISSENSCHAFT TECHNOLOGIE, vol. 97, no. 5, 1995, pages 194-199, XP000505134 the whole document</p>	1-10
A	<p>FARRELL D.: "The enrichment of poultry products with the omega n-3 polyunsaturated fatty acids : a selected review"</p> <p>PROCEEDINGS OF AUSTRALIAN POULTRY SCIENCE SYMPOSIUM, vol. 7, 1995, pages 16-22, XP000956231 the whole document</p>	1-10
A	<p>WO 97 10723 A (KEKES SZABO ANDRAS ;FARKAS TIBOR (HU); ATZEL EDWARD DE (HU); TOERO) 27 March 1997 (1997-03-27) page 2, line 23 -page 3, line 23; example 6</p>	1-10
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Inten I Application No  
PC1/ST 01/00084

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A	WO 95 21539 A (UNIV NEW ENGLAND ; FARRELL DAVID J (AU)) 17 August 1995 (1995-08-17) page 2, line 25 -page 5, line 22 -----	1-10
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A	US 5 897 890 A (SCHEIDELER SHEILA E) 27 April 1999 (1999-04-27) -----	

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